Spore dispersal of Endogonaceae by worms, ants, wasps, and birds

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Soil in earthworm casts (*Lumbricus terrestris* L.), ant castings (Formicidae), robin nests (*Turdus migratorius* L.), barn swallow nests (*Hirundo erythrogaster* Bodd.), and mud dauber wasp nests (Trypoxyloninae, Sphecinae) was examined for spores of Endogonaceae. Air-dried samples of worm casts, robin nests, or swallow nests added to steamed soil resulted in development of vesicular-arbuscular (VA) mycorrhizae in the roots of soybean (*Glycine max* (L.) Merr. 'Amsoy 71'). The results obtained indicate that organisms which transport soil may also disperse spores of Endogonaceae. In view of the large quantities of soil turned over by earthworms annually, these organisms may play a direct role in the vertical distribution of Endogonaceae in soil profiles. In addition through transport of castings by water runoff earthworms may play an indirect role in lateral distribution.

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Les auteurs ont recherché des spores d'Endogonacées dans le sol contenu dans des déjections de lombrics (*Lumbricus terrestris* L.), des rejets de fourmis (Formicidae), des nids de merle (*Turdus migratorius* L.), des nids d'hirondelle des granges (*Hirundo erythrogaster* Bodd.) et dans des nids de guêpes (Trypoxyloninae, Sphecinae). L'addition à du sol stérilisé à la vapeur d'échantillons séchés à l'air de déjections de lombrics et de nids de merle et d'hirondelle a mené au développement de mycorrhizes vésiculaires-arbusculaires dans les racines du soya (*Glycine max* (L.) Merr. 'Amsoy 71'). Les résultats montrent que les organismes qui transportent du sol peuvent aussi disséminer des spores d'Endogonacées. Considérant les grandes quantités de sol retournées annuellement par les lombrics, ces derniers peuvent jouer un rôle direct dans la distribution verticale des Endogonacées dans les profils de sol. De plus, les lombrics peuvent jouer un rôle indirect dans la distribution latérale des Endogonacées, par le biais du transport de leurs déjections par l'eau de ruissellement.

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Introduction

Reports dealing with dispersal of species of Endogonaceae indicate two known means of dispersal: (*1*) association with roots of plants which are transported, and (*2*) cotransport of spores and sporocarps with soil movement. As early as 1922, Thaxter (18) reported the occurrence of Endogonaceae spores in digestive tracts of millipedes. The ingestion and defecation of Endogonaceae spores by rodents have been investigated (1, 2, 6, 7, 8, 21). In many instances both stomach contents and feces have contained morphologically intact spores. Most rodent stomachs contained two or more species and one deer mouse contained five species of Endogonaceae (8). Trappe and Maser have germinated spores from the rectal area of *Microtus oregoni* (personal communication). Gerdemann and Trappe (8) have reviewed much of the literature dealing with animal and insect dispersal including rodents, grasshoppers, and crickets.

Investigations of the microorganismal constituents in the intestinal tracts of earthworms have failed to detect the presence of Endogonaceae spores or hypha (4, 5, 9, 14, 15, 19). In all instances axenic culture techniques were used and the presence of microorganisms was measured by growth on agar dilution plates. This failure can be attributed to the isolation techniques used in these investigations since the Endogonaceae have not been cultured on ordinary laboratory media (13). In light of this procedural deficiency in prior studies, the role of earthworms and several other organisms in spore dispersal was examined using living-host detection methods.

Materials and Methods

Extraction of Spores

Samples were collected in the vicinity of University Park, Pa., and recorded according to date (Table 1). Com-

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TABLE 1. Concentrations of Endogonaceae spores detected in soil associated with activities of various organisms

Sample	Collection date, mo./day/year	Total spores per gram soil[a]
Earthworm, *Lumbricus terrestris*[b]		
Casting 1	5/26/73	18.3
Check soil 1	"	55.9
Casting 2	"	9.4
Check soil 2	"	27.2
Casting 3	"	2.7
Check soil 3	"	1.8
Casting 4	"	2.1
Check soil 4	"	7.8
Casting 5	9/23/73	33.3
Check soil 5	"	35.0
Casting 6	"	44.0
Check soil 6	"	25.0
Casting 7	"	24.1
Check soil 7	"	39.0
Casting 8	"	21.0
Check soil 8	"	35.7
Ant, Formicidae[b]		
Casting 1	5/26/73	84.9
Casting 2	"	40.4
Casting 3	"	16.3
Casting 4	"	31.6
Casting 5	"	26.5
Casting 6	"	73.4
Casting 7	"	5.2
Robin, *Turdus migratorius*		
Nest 1	6/26/73	4.6
Nest 2	"	3.7
Swallow, *Hirundo erythrogaster*		
Nest 1	9/22/73	36.3
Nest 2	"	18.6
Nest 3	"	28.7
Wasp, Trypoxyloninae		
Nest 1	10/13/73	5.9
Wasp, Sphecinae		
Nest 2	10/13/73	12.9

[a]Earthworm casting soil based on air-dry weight. All others based on ovendry sample weight.
[b]Check soil samples of a 0- to 10-cm-depth profile were taken in the immediate area of the worm or ant casting. Ant castings 1–4 are comparable in location to worm casting 1 and check soil 1, ant casting 5 to check soil 2, ant casting 6 to check soil 3, ant casting 7 to check soil 4.

plete bird nests or wasp nests which were occupied in the current year were collected individually and placed in polyethylene bags. Worm casts and ant casting materials were carefully collected to avoid contamination of the samples with the underlying soil. Check soil samples of a 0- to 10-cm-depth profile were taken with a 2-cm-diameter soil sampling tube in the area immediately adjacent to the worm cast mounds. Spores were extracted from portions of the samples according to the method of Sutton and Barron (17). Spore counts were made for each sample.

Earthworms (*Lumbricus terrestris* L.) were also collected at worm cast locations (Table 1). In the laboratory, the worms were sacrified by immersion in hot water and were dissected, and the intestinal contents were examined for the presence of Endogonaceae spores.

Formation of VA Mycorrhizae

Ten grams of crushed air-dried sample material was mixed with about 400 g of Hagerstown loam and placed in plastic containers. The loam soil had been treated previously with aerated steam at 65 °C for 30 min to kill any Endogonaceae spores present in the soil. The sample materials included worm casts, robin nests, and swallow nests. The check treatments consisted of steamed soil with no additional material. 'Amsoy 71' soybean seed were planted in the prepared soil and thinned to three plants per container after emergence. Each treatment was replicated three times. The plants were grown in the greenhouse for 4 weeks. The root systems were then carefully washed free of soil and 25 root segments, each 1 cm long, were cleared in 10% KOH (16). The root segments were then stained with trypan blue in lactophenol and mounted on microscope slides, and the percentage of root infection by vesicular-arbuscular (VA) mycorrhizae was determined according to the method of Hayman (10).

Results

Isolation of Spores

Spores were found in all samples examined (Table 1). In general, more spores were extracted from worm casts than from corresponding check samples. Spore concentrations in some ant casting samples were not related to corresponding check samples (ant castings 1, 3, 6) while in others the spore concentrations were similar in both (ant castings 2, 5, 7).

Relatively few spores were extracted from the small amount of soil contained in the robin nests. In contrast, swallow nests, consisting largely of soil, contained considerably greater numbers of spores. Wasp nests contained low numbers of spores. Intestinal contents of all worms examined contained Endogonaceae spores.

Formation of Endotrophic Mycorrhizae

VA mycorrhizae did not develop in roots of soybean plants grown in steam-treated check soils. However, in soils amended with air-dried worm cast material, soybean roots contained 1.53 to 3.80% VAM infection on a length-of-root-infected basis. The low number of spores in the robin nest material might account for the low percentage root infection. However, the swallow nest treatments developed few mycorrhizae (0.0 to 0.4% infection) despite the relatively high numbers of spores which could be extracted from nest materials (Table 2).

Discussion

The results obtained in the present study with earthworms, bird nests, and wasp nests com-

TABLE 2. Percentage root infection of Amsoy 71 soybean by VA mycorrhiza after 4 weeks growth in steamed soil infested with worm cast or bird nest material

Inoculum[a]	Percentage root infection
Worm cast 5	1.5
Worm cast 6	3.8
Worm cast 7	2.4
Worm cast 8	1.7
Robin nest 2	0.9
Swallow nest 1	0.0
Swallow nest 2	0.4
Swallow nest 3	0.3
Check 1	0.0
Check 2	0.0
Check 3	0.0

[a]Air-dried inoculum material (10 g) was mixed in about 400 g of steamed loam soil.

bined with the results of Gerdemann and Trappe (8) and Trappe and Maser (personal communication) with small rodents suggested strongly that various living organisms are involved in distribution and dissemination of Endogonaceae.

Darwin (12) has estimated that earthworm activity could bring as much as 2 to 5 cm of soil to the surface in a decade and that the quantity of soil which passes through the digestive tract of these animals annually would amount to 0.367 kg m^{-2} of dry earth. This large amount of soil which is constantly being mixed through earthworm activity is probably an important means of distribution of Endogonaceae spores within the soil. Our results show that the spores which have passed through the earthworm are still capable of forming VA mycorrhizae. The dispersal of certain soil fungi through earthworm activity was reported by Hutchinson and Kamel (9) and by Thornton (19). The methods used in their investigations did not allow the detection of Endogonaceae and therefore an important part of the ecological significance of the earthworm remained unrecognized.

Ants may be less important than the earthworm in the dispersal of Endogonaceae since these insects would transport less soil. Prairie ants have been estimated to bring soil to the surface at the rate of 2.5 cm in 500 years (3) or about $\frac{1}{50}$ of the rate of earthworm transport. Although our sampling was rather limited, in nearly every case the spore concentrations found in ant casting material was greater than those found in worm casts taken from the same area. This observation would indicate that the possible relative importance of these two animals in the dispersal of Endogonaceae spores should not be measured by the relative amounts of soil moved by each over equal periods of time.

Warner and French (20) isolated many genera of culturable fungi from wild birds, but the techniques used did not involve soil moved by birds or the detection of Endogonaceae. At least two of the swallow species which they examined nest in cliffs (11) and could conceivably come into contact with various soils infested with Endogonaceae which the birds could then disperse over other areas. The importance of birds and mud dauber wasps in dispersal of the fungus is probably rather limited in view of the relatively small amounts of soil transported by these agents; however, the distance and speed aspects of these agents should not be overlooked.

It appears to us that ants and earthworms bring Endogonaceae spores to the surface. These organisms' movements may result in some horizontal dispersion, a few metres at most. The major long-distance transport would occur through wind or water erosion of the ant or earthworm castings.

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